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## Detection of Cytomegalovirus, Rubella virus, and IL-2 Levels in a Sample of Recurrently Aborted Iraqi Women

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### Abstract

The present study aimed to investigate CMV and Rubella virus as a causative agent of recurrent abortion, while the IL-2 levels were estimated as immune parameter during pregnancy period. A total of 63 blood samples were collected from recurrently aborted women, control non-pregnant women and control pregnant women. The results recorded 72.09 % CMV positive aborted women and 27.91 % Rubella virus positive aborted women. Levels of IL-2 were  $(437.03 \pm 38.02)$  pg/ml in first group,  $(390.51 \pm 63.56)$  pg/ml in second group,  $(32.98 \pm 15.12)$  pg/ml in control group non pregnant women and  $(118.63 \pm 24.81)$  pg/ml in control pregnant women. High IL-2 levels in all studied women indicate presence of a factor affecting the immune system other than viral infection or pregnancy which may be needing more investigations.

**Keywords:** Cytomegalovirus, Rubella virus, Interleukin-2 and Iraqi women.

## الكشف عن الفيروس المضخم للخلايا و فيروس الحميراء ومستويات IL-2 في عينة من نساء الإسقاط المتكرر في العراق

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### الخلاصة

هدف البحث الحالي إلى التحري عن فيروس المضخم للخلايا و فيروس الحميراء (الحصبة الألمانية) كمسببات للإسقاط المتكرر عند النساء وتم قياس IL-2 مؤشرا مناعيا . تم جمع 63 عينة دم من نساء الإجهاض المتكرر ونساء السيطرة غير الحوامل ونساء السيطرة من الحوامل ، بينت النتائج وجود فيروس المضخم للخلايا في 72.09% من النساء المسقطات ووجود فيروس الحميراء في 27.91% وكان معدل مستويات IL-2  $(437.03 \pm 38.02)$  في المجموعة الأولى،  $(390.51 \pm 63.56)$  في الثانية و  $(32.98 \pm 15.12)$  في نساء السيطرة الغير الحوامل و  $(118.63 \pm 24.81)$  في نساء السيطرة الحوامل . يؤثر المعدل العالي للمجاميع الأربعة أن هناك عامل أو عوامل أخرى مؤثرة على الجهاز المناعي غير الإصابات الفيروسية أو الحمل الشيء الذي يستوجب مزيدا من التحري.

### Introduction

Recurrent spontaneous abortion (RSA) is one of the most common obstetrical complications. It has been shown that the total rate of spontaneous pregnancy loss is 31.0% out of the total abortion cases [1]. The majority of pregnancy losses are random or isolated incidences that in many cases are related to genetic abnormalities [2]. RSA due to maternal infections transmissible in uterus at various stage

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of gestation can be caused by a wide array of organisms which include (Rubella virus, Cytomegalovirus)[3,4].

Infection with human cytomegalovirus (CMV) is very common world-wide with seropositivity rates ranging from 40% in developed countries up to 100% in developing countries [5,6]. The CMV, which can produce chronic or recurrent maternal infection and exhibits a high tropism for cervical mucosa, is the most implicated virus as a potential cause of RSA. During pregnancy, in fact, CMV can reach the placenta from the cervix or by viremia following both primary and recurrent maternal infection, with subsequent vascular insufficiency, tissue damage, and transmission to the fetus[7,8]. Rubella is a common cause of maculopapular rash illness with fever. The disease has few complications unless it is contracted by a pregnant woman especially in the early weeks of gestation. Rubella infection in pregnancy can lead to miscarriage, stillbirth, or an infant born with congenital rubella infection [9,10].

IL-2 is a very important Inflammatory cytokine, Abnormalities of IL-2 production have been found in patients undergoing transplant rejection [11,12]. A lot of studies have been shown that the higher levels of circulating IL-2 were related to RSA [13]. Hence, our study aimed to investigate CMV and Rubella virus as a causative agent of recurrent abortion, while the IL-2 levels were estimated as immune parameter during pregnancy period.

## Materials and Methods:

### Studied group

A total of 63 blood samples were collected from woman Obstetrics and Gynecology Department of Al-Yarmouk Teaching Hospital, Baghdad Teaching Hospital, Fatima Al-Zahra'a for Obstetrics and Children Hospital, and Al Alwaiya Teaching Hospital in Baghdad. Patients' ages ranged between (18-45) years during this study (samples collection started during Feb. 2014 and lasted until June 2014). Four different groups of women used in this study:

1. Group one (G1): Included 10 non pregnant healthy control women.
2. Group two(G2): Included 10 women with successful pregnancy (normal delivery) as control group
3. Group three (G3): Included 43 women admitted to the hospital for recurrent spontaneous abortion (3-6 numbers of abortions) for evacuation.

### Serum separation

Three milliliters (3ml) of venous blood were taken by venipuncture from all patients and healthy subjects included in this study. Three milliliter (3ml) was put in vacuumed tube with gel (Without anticoagulant) and left at room temperature until the blood clotted; then serum was separated by centrifugation for 10 min at 2500 r.p.m, stored at -20°C in the deep freezer.

### Estimation of IL-2 concentration

Estimation of IL-2 level in serum was done by ELISA method. According to using Bostar Immunoleader ELISA kit (USA).

### Serological diagnosis of CMV and Rubella virus

#### 1- OnSite CMV and Rubella virus IgG / IgM Rapid Test-Cassette.

The test was done according to [14]

#### 2- ELISA technique

Samples of serum were screened for the presence of IgM and IgG antibodies against (Rubella virus and Cytomegalovirus) by ELISA kit from Bio.check, Ink, (USA).

### Statistical analysis

Chi-square test was used to significant compare between percentages and least significant difference –LSD test was used to significant compare between means in this study. Values were considered statistically significant  $P \leq 0.05$ .

## Results and discussion

The results of present study recorded CMV antibodies in sera of 31 out of 43 aborted women (72.09 %) which indicate that CMV is the major causative agent for recurrent spontaneous abortion as compared with Rubella virus antibodies which diagnosed in 12 aborted women (27.91 %) involved in the present study. The statistical analysis of the positive results of CMV showed highly significant difference, ( $P \leq 0.01$ ) compared with Rubella virus showed significant difference, ( $P \leq 0.05$ ) Table-1.

Results of our study showed increase in CMV percentages as compared with other local studies where they were 21% in Baghdad[15] and in Iraqi Governorates, 90.4% of aborted women in Al-Anbar Governorate, 60% in Thiqr Governorate [16,17] .

**Table 1-**percentages of CMV, and Rubella virus of aborted women

Causes	Patients		Chi-square- $\chi^2$
	No.	Percentages (%)	
CMV	31	72.09	7.631 **
Rubella V.	12	27.91	5.065 *
Total	43	100 %	----
Chi-square- $\chi^2$	---	11.207 **	---
* (P≤0.05), ** (P≤0.01)			

In Arabian countries CMV positive aborted women percentages were reported 96.6 % in Tunisia,63.3% in Saudi Arabia [18,19]. Other reports from different countries recorded CMV positive aborted women55.3 % in India [20] and 97%in Brazil [21].

The results of present study agree with other local study on aborted women in Baghdad where susceptibility rate of Abs positive Rubella virus was 20.6 % [22] while it differ with results of other one where percentages of Rubella virus positive was 34.2 % [4]. In Iraqi Governorates Rubella virus antibody disagree recorded as 16 % in Mosul, 78.33% in Babylon [23,24].

In Arabian countries it was reported as, 6.7 % in Saudi Arabia, 34.7% in Sudan [25,26]. Other reports from different countries recorded 24 % in SriLanka [27], 21.1% in aborted women in india [28].

The lower percentages of Rubella infection in aborted women might be explain according to educational level of the patient women where nine out of twelve women in present study had primary and intermediate education, as conclusion these Iraqi women were not immunized against Rubella since Rubella vaccination is done according to WHO program and it is needed by the Iraqi female for registration at secondary school [29].

### Estimation of IL-2

IL-2 levels in different studies groups were demonstrated as shown in table-2.

**Table 2-** IL-2 levels (pg/ml) in sera of studies groups

Group	No.	Mean ± SE of IL-2
Control non-pregnant	10	32.98 ± 15.12
Control pregnant	10	118.63 ± 24.81
Patients (aborted)	43	380.23 ± 267.63
LSD value	----	102.47 **
P-value	----	0.0001
** (P≤0.01)		

In control non pregnant women IL-2 value range from -26.6 to 135pg/ml with mean value of (32.98 ± 15.12)and from 10 to 261.6 pg/ml with mean value of (118.63 ± 24.81) in control pregnant women while IL-2 value in aborted women range from -15 to 935 pg/ml with mean value of (380.23 ± 267.63) . Other local studies recorded mean value of IL-2 levels in aborted women as 259.32± 82.6 [30] and 349.5 ± 13.2 [31].

The IL-2 in control non pregnant women in table-2 means value of (32.98 ± 15.12). In a study the normal value of healthy control of IL-2 is rang from (0 – 15) pg/ml [32, 33]. Elevated serum levels of IL-2 have been detected in several pathologic conditions, including hemopoietic malignancies, solid neoplasms, and autoimmune and infectious diseases [34,35].

Also IL-2 in pregnant women mean value of  $(118.63 \pm 24.81 \text{ pg/ml})$ , other study in Baghdad recorded mean value of IL-2 levels in control pregnant women as  $(97.45 \pm 0.90 \text{ pg/ml})$ [30], other studies in Kuwait demonstrated concentration of IL-2 was slightly higher in normal pregnant women  $(76.3 \pm 13.7 \text{ pg/ml})$ [36], other report suggests elevated concentrations of IL-2 in normal pregnancy as compared with non-pregnant controls [37,38]. It is tempting to suggest that IL-2 may be another immunologic marker for the investigation of pathologic pregnancy [37].

The present study result of IL-2 levels recorded three minus results (-8.33, -26.6, -15 pg / ml). The Low IL-2 serum levels in women with recurrent miscarriage probably may be associated with a lack of T and B-cell growth promoting effects which in its turn lead to insufficient induction of a pregnancy protecting maternal immune response. The possible cause of low IL-2 serum levels could be reduced maternal immunization against paternal antigens [11, 39].

The elevated IL-2 levels in control and patient subject beside the minus results -8.33, -26.6, - 15 indicate presence of factor other than abortion, viral infection and pregnancy causing the above abnormal results. A fact that may needing more investigations.

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